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EFFECT OF DIFFERENT ENVIRONMENTAL FACTORS ON AMYLOLYTIC ACTIVITY OF *Bacillus cereus* AND *Bacillus mycoides*

WPLYW RÓŻNYCH CZYNNIKÓW ŚRODOWISKOWYCH NA AKTYWNOŚĆ AMYLOLITYCZNA *Bacillus cereus* I *Bacillus mycoides*

Abstract: The influence of different factors on amylolytic activity *Bacillus cereus* (2 strains) and *Bacillus mycoides* (2 strains), isolated from soil samples and water of Turawa Lake has been studied. Effect of physiological (temperature of reaction from 30 to 60 and pH of reaction mixture in the range of 4.0÷8.0) and nutritional parameters (the carbon sources and they concentration in medium) were examined spectrophotometrically for their effect on amylase production. The amylase activity was estimated on the basis of the reduction in blue colour intensity resulting from enzymatic hydrolysis of starch. The results obtained, that in view of all studied factors, the best pH level appear 7.0 or 8.0 and temperature 60°C for all tested strains. Moreover amylase yield was the highest in media containing maltose or glucose as sole carbon source in all tested temperature.

Keywords: *Bacillus cereus*, *Bacillus mycoides*, amylolytic activity, pH, temperature

Bacillus spp. play an important role in changes occurring in nature and are often used in technological processes due to they ability to degradation of substrates complex. Industrial application of these microorganisms has been investigate extensively and some of their extracellular enzymes such as amylases, cellulases, pectinases, proteinases have been put to use on an industrial scale [1].

Amylases are among the most important hydrolytic enzymes for all starch based industries. In present day, they find application in all the industrial processes, such as in food, textile, detergent, pharmaceutical, paper, brewing and distilling industries, agriculture and environment protection for the hydrolysis of starch [2-4]. Production of enzymatic preparations using these bacteria is one of the most important branchens of biotechnology.

However, according to data from literature the *Bacillus* spp. behaved differently in terms of their enzymatic activity. The efficient production of *Bacillus* amylases depends on several physical and chemical factors, such as pH of reaction mixture, temperature of mixture, composition of the cultivation medium (carbon and nitrogen sources), period incubation, moisture and agitation. Interactions of these parameters are reported to have a significant influence on the productive synthesis of the enzymes [5-8].

The aim of presented research was the evaluation of the ability to synthesise amylases by selected *Bacillus* strains depending on the choice parameters.

Materials and methods

The bacterial strains used in this study were *Bacillus* species, previously isolated from soil samples (2 strains marked as *Bacillus cereus* A96 and *Bacillus mycoides* A134) and water of Turawa lake (2 strains marked as *Bacillus cereus* G10 and *Bacillus mycoides* G3).

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Amylase *Bacillus* species were screened in this study by using starch hydrolysis procedure and following cultivated for 48 h at 30°C on growth media enriched in: potato starch (P), corn starch (K) or maltose (M) in the range of 1% to 5% and glucose (G) - 1%.

Enzyme activity of the supernatant after removal of the bacteria by centrifugation the media at 4000 rpm for 20 min was tested. The effect of pH on amylase activity was investigated by measuring enzyme activity at 37°C in different buffer solution (0.1M) (pH values from 5.0 to 8.0), whereas the effect of temperature was evaluated by incubating the reaction mixtures at different temperatures in the range of 30÷60°C. The amylase activity was estimated on the basis of the reduction in blue colour intensity resulting from enzymatic hydrolysis of soluble starch (0.2%) as substrate according to the starch-iodine modified Fennela method. One unit enzyme activity was defined as the quantity of enzyme that causes 1% reduction colour intensity of starch-iodine solution in 30 min (U/cm³/30 min).

Results and discussion

The addition of carbon source in the form of either monosaccharides or polysaccharides could influence the production of enzymes. Potato starch, corn starch, maltose are major substrate considered for enzyme production in this study. The effects of different factors (pH, temperature) and carbon sources on amylase production showed that each bacteria behaved differently.

In the dependent on strains and medium degree of starch decompose was different in the range pH value from 5.0 to 8.0. Soil occurring strains, particular *Bacillus cereus* A96 show the highest activity at pH value 5.0. The growth maltose medium turned out to be a favourable medium for its. The enzyme activity reached from 63.16 to 84.21 units (U/cm³/30 min). However, the lowest amylase activity was found in case of water occurring strains, where *Bacillus mycoides* G3 did not amylase produce at pH 5.0. Non of the strains tested did not show enzymatic activity at glucose as carbon source (Fig. 1).

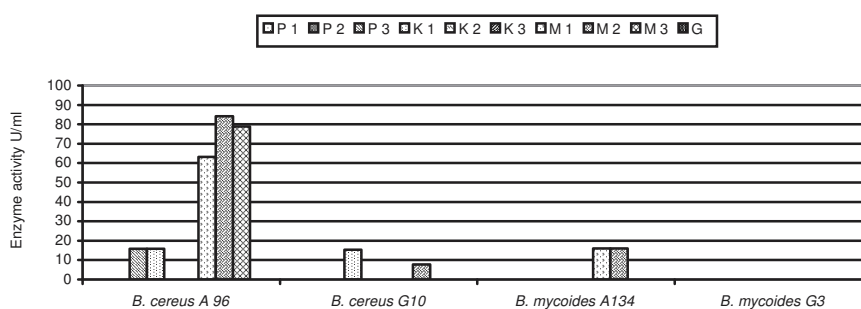


Fig. 1. Effect of pH 5.0 on amylase activity of *Bacillus cereus* and *Bacillus mycoides*

The increase pH value for enzyme reaction resulted in an increasing amylolytic activity of water occurring strains (data not shown), but at following pH values (7.0, 8.0) the *Bacillus* spp. demonstrate different maximum activity at particular media depending on carbon sources (Fig. 2).

The results obtained at pH 7.0 showed that most of *Bacillus* spp. are able to produce considerable amount of amylase on most of the media.

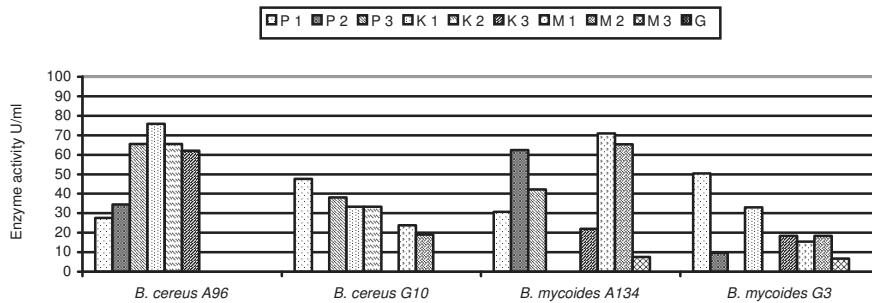


Fig. 2. Effect of pH 7.0 on amylase activity of *Bacillus cereus* and *Bacillus mycoides*

Soil occurring bacteria were found to be most vigorous strains for the highest amylase activity. The *Bacillus cereus* A96 and *Bacillus mycoides* A134 presented higher enzyme yield in potato starch production media, from 27.59 to 62.52 and 30.63 to 62.43, respectively. Besides, *Bacillus cereus* A96 showed the highest enzymatic activity in corn starch media while *Bacillus mycoides* A134 in maltose media. The results related to corn sources showed that non of the strains did not produce enzymes in glucose media, it was similarly like at pH value 5.0. On the basis of the obtained results, the highest ability for the amylase synthesis was found for *Bacillus cereus* A96 and *Bacillus mycoides* A134 strains.

The supernatant amylolytic activity was assayed at different temperatures at a constant pH pf 7.0 and a substrate concentration of 0.2% (starch solution in phosphate buffer). The results obtained in our study showed, that the activity of amylase increased with increasing temperature starting from 30°C till reached to the optimum activity at 60°C. The minimum level of amylase occurred at 30°C as shown in the Figure 3.

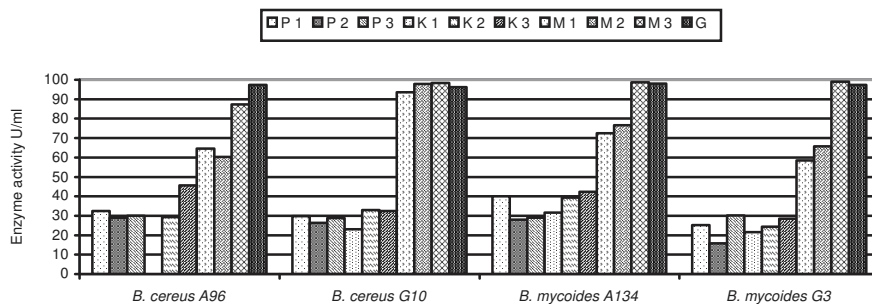


Fig. 3. Effect of temperature 30°C on amylase activity of *Bacillus cereus* and *Bacillus mycoides*

Among from all the strains, *Bacillus cereus* marked A96 was the most activity in potato starch or glucose production media, but *Bacillus cereus* marked G10 in media with corn starch or maltose. However the highest yield of amylase enzyme was obtained for *Bacillus*

mycooides A134 in all tested media. The maximum potato starch concentration in medium for the optimal amylase production for all bacteria was 1%, while similar effect was found with increasing corn starch and maltose concentration in medium to 5%. The enzymatic activity level in medium with glucose was approach to obtained in medium containing 5% maltose. Soil occurring strains *Bacillus cereus A134* and *Bacillus mycooides A134* were the most active, whereas *Bacillus mycooides G3* the least active. In extracellular amylases biosynthesis the most effective source of carbon was maltose in this temperature. The optimal temperature for production of amylase of all *Bacillus* spp. occurred at 60°C where they got the maximum activity, particularly *Bacillus cereus G10* and *Bacillus mycooides A134*. Either strains were the most active in all tested media (Fig. 4).

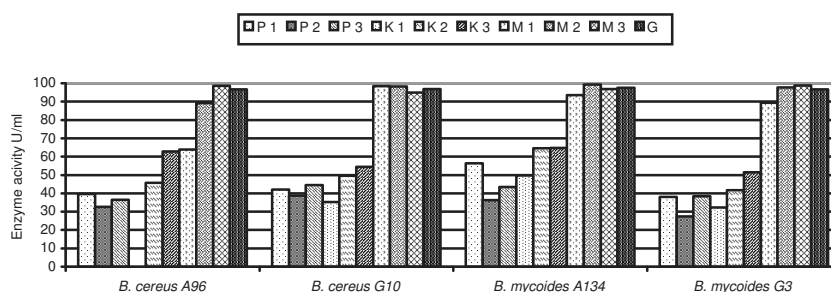


Fig. 4. Effect of temperature 60°C on amylase activity of *Bacillus cereus* and *Bacillus mycooides*

Soil occurring strains preferred potato medium at lower concentration (1%) when water occurring strains at higher concentration (5%). Moreover the all tested strains preferred medium containing 5% corn starch. In the presence maltose or glucose in medium as carbon sources the highest amylase yield was obtained from all strains, similar to that at 30°C. Among of all the strains, *Bacillus mycooides A134* was found to be most efficient whereas *Bacillus mycooides G3* the least for the production of amylase at this temperature.

Conclusions

Conducted research proved significant diversity between particular *Bacillus* strains in terms of their amylase production, when different factors such as pH, temperature and media composition were applied and enabled to draw the conclusion:

1. Biosynthesis of amylase catalysed by *Bacillus* spp. at different pH was the most diversified in depending of strain and production media. But in majority of events the best pH level appears 7.0.
2. In view of temperature the most effective strain was *Bacillus mycooides A134* whereas the least *Bacillus mycooides G3*.
3. The highest level of amylase yield was obtained at 60°C and the production medium with maltose or glucose was profitable in the best for all tested strains.

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Abstrakt: Celem badań była ocena wpływu różnych czynników na aktywność amylolityczną *Bacillus cereus* (2 szczepy) i *Bacillus mycoides* (2 szczepy), wyizolowanych z gleby i jeziora Turawa. Badano wpływ pH mieszaniny reakcyjnej w zakresie od 5,0 do 8,0 i temperatury w zakresie od 30 do 60°C oraz źródła węgla i jego koncentracji w podłożu na poziom amylaz. Na podstawie stopnia zmniejszenia się zabarwienia z jodem oznaczono ilość rozłożonej skrobi metodą spektrofotometryczną. Uzyskane wyniki badań wykazały, iż spośród przebadanych czynników dla wszystkich szczepów najlepszą wartością pH była 7,0 lub 8,0, a temperatura 60°C. Ponadto najwyższy poziom amylaz uzyskano, hodując badane szczepy na podłożu z maltozą lub glukozą w zakresie badanych temperatur.

Słowa kluczowe: *Bacillus cereus*, *Bacillus mycoides*, aktywność amylolityczna, pH, temperatura